PROTECTIVE EFFECT OF GRAPE SEED EXTRACT IN EXPERIMENTAL MENOPAUSAL SYNDROME

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PROTECTIVE EFFECT OF GRAPE SEED EXTRACT IN EXPERIMENTAL MENOPAUSAL SYNDROME (Abstract) Grape seed extract (GSE) is a natural product obtained from Vitis vinifera seeds; it has been used as adjuvant therapy in wide range of pathological conditions. **Aim:** In the present study, we investigated the ability of GSE to improve the mental status on ovariectomized rats. **Material and methods:** GSE (10 mg/kg/day, 60 days long) was administrated orally on ovariectomized adult female rats; then the animals were investigated in open field. Additionally, were measured the hematological parameters, the plasma biochemistry, and the oxidative stress markers. **Results and discussion:** Treatment of GSE improved the loco motor activity in the open field, the number of crossings were almost twice in GSE treated rats (22.25±8.75) compared to placebo group (13.00±2.62) (p<0.05). Lipid peroxides were reduced up to 51.57% (p<0.05), while hematology and plasma biochemistry remained almost unchanged. **Conclusion:** GSE improve the mental status and reduced the level of oxidative stress in rats subject to experimental ovariectomy. **Keywords:** POLYPHENOLS, OXIDATIVE STRESS, OVARIECTOMY, OPEN FIELD

Menopause is the cessation of spontaneous menstrual cycling; the average age of menopause in women is approximately 50 years, resulting a post reproductive period for one third of their lives. A number of other physiological systems are also affected by the sudden withdrawal of hormonal support associated with menopause, including bone density, cardiovascular health, cognition and possibly some cancers. Finding an appropriate model is a debated subject, but the use of rodent models is manifold, because they have a relatively low life span, ovarian function is well characterized and homogenous laboratory strains allowed controlled experiments (1). This experimental model is widely used to prove the efficiency of various plant compounds against menopause associated pathology; polyphenols from green tea proved to have a protective effect against bone loss in on ovariectomized female rats in non dose dependent manner, the same osteprotective effect was provided by phytoestrogens like coumestrol and zearalanol (2); on the other hand, soy isoflavone prevent oxidative changes in hepatocytes (3) and flavones from Radix puerariae reduce the hepatic
accumulation of triglycerides (4).

Grape seed extract (GSE) is a natural product obtained from Viis vinifera seeds; and they are a byproduct of wine. Nowadays, the interest for GSE is fully justified, they contain polyphenols, mainly flavonoids, proanthocyanidins, galic acid, cathechins etc. The most important constituents are the polyphenoles, which act not only as effective free radical scavenges, but they modulate de activity of antioxidant enzymes, block hormone receptors, and reduced the activity of various mutagens. Experimental and clinical studies show that polyphenols protect the blood vessel, prevent the platelet aggregation, and lower the LDL-cholesterol.

MATERIAL AND METHODS

Nutritive supplement. Grape seed extract (GSE) was a commercial capsulated product 100 mg / capsule, a generous gift of TC Alsifcom Ltd. The hydro soluble premix (extracted with hydro alcoholic solutions), was manufactured by Eurochem Feinchemie Gmbh (batch number 4001103K001). The analysis bulletin was performed by high performance liquid chromatography (HPLC); its composition was as follow catechins 2-3%, epicatechins 2-3 %, proanthocyanidin oligomers (bi- and trimetric) 6-9%, proanthocyanidin (polymers) 89 %, total of proanthocyanidin oligomers were over 92%. The product was within acceptable range of microbiological contamination and residues. The animals in study received 10 mg/kg/day, dissolved in water, immediately before administration, for all experiment long.

Animals. Experiments and animals welfare were according to the Guide for the Care and Use of Laboratory Animals (Department of Health Education, and Welfare, National Institute of Health, 1996), and followed the guidelines of European Communities Council Directive (86/609/1986) and Ordinance No. 37 of the Romanian Government from 2nd February 2002. The animal tests and experiments were allowed by the Bioethical Board of the Faculty of Veterinary Medicine Cluj-Napoca. The animals were caged in polycarbonate cages, at controlled temperature of 21-22ºC, humidity (40-60%) and 12/12h light/dark cycle. Standard lab chow, soy free (to avoid any source of estrogens), provided by National Institute for Research and Development Cantacuzino Bucharest, and water were freely available.

Experimental protocol. Experiment was carried out on 24 albino Wistar female rats, 232.75±8.52 g body weight, divided into tree equal groups, of 8 animals each. Groups I was the sham operated group, it represents the control. Groups II and III were subject to ovariectomy, and, additionally, 5 days after surgery, group 3 received GSE therapies orally, by using a lubricated catheter, daily, for 60 days long, until the end of the study. The other two groups received isotonic saline solution as placebo. In the end, the animals were subject to open field test; blood samples were drawn under deep narcosis and, than, euthanasia was made. Immediate latter, the animals were subject to gross examination and uterus weight was determined.

Surgical procedure. The surgery was performed under isoflurane narcosis, supplemented by neuroleptanalgesia by xylazine 0.2% (Narcoxl) 0.2 ml/kg and ketamin 10% 0.3 ml/kg injected intramuscularly. A longitudinal incision was made along vetromedian area, between umbilical region and pubis; the ovaries were exteriorized, and excised. Abdominal muscles and skin were sutured with Dexon and polyacrilate respectively. The sham operated
animals were subject to laparotomy, but whiteout removal of the ovaries. The subjects were allowed 5 days to recover from surgery before starting the experiments.

Open Field Test. Open field test was run within in a box measuring 60x40x50 cm, whose floor was divided by lines into 12 equal squares. Animals were placed facing the rear left corner of the arena and observed for 3 min. Number of squares crossed with the four paws from one square to another was indicative of motor activity. Measuring score included defecation, urination and ambulations.

Laboratory analysis. Blood hematology was investigated immediately, using EDTA anticoagulated blood, with Abacus Junior Vet, automatic analyzer Diatron Messtechnik, Budapest, Hungary. For biochemical analysis, the blood drawn in clot activator vacutainers, was centrifuged at 2000 rpm for 10 min to separate the serum, immediately after separation serum samples were frozen at – 20 °C. Blood chemistry (cholesterol, triglyceride, alkaline phosphatase etc.) was measured using screen point semiautomatic analyzer, STAT – FAX 1904 Plus, Global Medical Instrumentation, Inc. 6511 Bunker Lake Blvd. Ramsey Minnesota, 55303 USA, by using special determination kits.

Lipid peroxides were determined, by measuring the production of tiobarbituric reactive substances according to the method of Burge and Aust, protein carbonyl were evaluated by using guanidine chloride. Ravin method (parafenildiamin dichloride) was the choice for measuring the plasma ceruloplasmin, while Hatano (diphenyl picrichydrol) method was preferred for measuring hydrogen donor ability.

Statistics. All data are reported as the mean ± SEM. The Gaussian distribution was checked by Shapiro-Wilk normality test. One-way analysis of variance ANOVA, followed by post hoc Dunnett’s range test procedure was done Statistical significance was at p<0.05 (95% confidence interval). Statistical values were obtained using GraphPad Prism version 5.0 for Windows, GraphPad Software, San Diego California USA.

RESULTS AND DISCUSSION

The mean body mass increased significantly in both ovariectomized groups (II and III) compared to group I (p<0.05), body weight gain was associated with high amount of abdominal fat. Increasing in body weight and abdominal fat accumulation is a widely accepted side effect of ovariectomy as a consequence of sudden drop in estrogen level (4). Expectedly, the uteri of rats from groups II and III revealed severe atrophy, highly significant as compared to group I (p<0.001). GSE therapy has no influence the body weight or the degree of uterus involut, therefore GSE does not seem to exert any estrogenic effect on the genital organs or other body areas (tab. I).

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Variation of body weight and uterus weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>b.w. initial (g)</td>
</tr>
<tr>
<td>Control</td>
<td>215.20±18.09</td>
</tr>
<tr>
<td>Ovariectomy</td>
<td>248.75±06.30</td>
</tr>
<tr>
<td>Ovariectomy +GSE</td>
<td>177.33+11.45</td>
</tr>
</tbody>
</table>

Result expressed as mean ± SD, b.w. = body weight. GSE = Grape seed extract
* and ** = statistically significant as compared to Control group at p<0.05 and p<0.001
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Oxidative stress markers were increased following ovariectomy, but significant results we found only for malondialdehyde (p<0.05). The therapy was effective, the values in group III were significant lower than in group II (p<0.05), and they were similar to group I rats. In menopausal women, is already proved a clear relationship between the plasma levels of malondialdehyde and the logarithm of the plasma estradiol concentrations, which was best demonstrated with a quadratic regression (5), estrogens having a well known antioxidant protective effect. The antioxidant protective effect of GSE is not surprising; it was already proved on various experimental models (tab. II).

**TABLE II**

<table>
<thead>
<tr>
<th></th>
<th>Malondialdehyde (nmol/ml)</th>
<th>Protein carbonyl (nmol/ml)</th>
<th>Hydrogen donors ability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.8±1.74</td>
<td>1.54±0.76</td>
<td>31.06±6.95</td>
</tr>
<tr>
<td>Ovariectomy</td>
<td>11.13±2.95*</td>
<td>1.20±0.35</td>
<td>34.66±10.13</td>
</tr>
<tr>
<td>Ovariectomy +GSE</td>
<td>5.74±1.29†</td>
<td>0.80±0.15</td>
<td>28.24±8.66</td>
</tr>
</tbody>
</table>

Result expressed as mean ± SD, GSE = grape seed extract
* = statistically significant as compared to Control group at p<0.05
† = statistically significant as compared to Ovariectomy group at p<0.05

Plasma biochemistry showed increase serum alkaline phosphatase (AP) activity in both groups II and III (184.7±73.5 u/l, and 164.7±53.3 u/l, respectively) as compared to I (137.8±37.2 u/l,) (p<0.05). The relation between the loss of bone density and elevated activity for AP is widely accepted; so, antosteoporotic therapy also down regulates the AP activity (2). Similar values of PA between groups II and III suggest that GSE failed to prevent the bone destruction. Other blood enzymes (GGT, ASAT and ALAT) reveal no difference among groups in study, so were the other biochemical compounds (triglycerides, cholesterol, urea, creatinine, glucose, total bilirubin, total protein, and calcium). Hematology was also, within normal limits for all groups. In female Guinea pigs, subject of ovariectomy and a high cholesterol diet, the GSE supplementation reduced the plasma triglycerides. Even that cholesterol levels remained high, the VLDL cholesterol was diminished, and, by down regulation of cholesterol acyle transferase, GSE prevents the cholesterol accumulation into the aortic walls (6). Furthermore, GSE proanthocyanidins prevent the posprandial peroxidation of lipids, and peroxidation of LDL (7). Clinical studies also confirm the protective effect of GSE against menopausal associated dyslipidemia; it reduced the plasma triglycerides and LDL, by diminishing the systemic inflammatory status and oxidative stress (8). In our findings, the ovariectomy was not associated with any obvious impairment of excretory, liver or metabolic function, probably because the animals received a balanced diet, whiteout atherogenic compounds, which unrevealed a possible protective effect of GSE on lipid metabolism. Our findings correlated to literature data, suggest once again that, in addition to hormonal imbalance, dyslipidemia associated with menopausal syndrome have an important alimentary etiology.
**TABLE III**

**Results of the Open field test**

<table>
<thead>
<tr>
<th></th>
<th>N. of rearing</th>
<th>No. of defecations</th>
<th>No. of crossings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>7.75±2.92</td>
<td>3.13±1.13</td>
<td>27.00±7.45</td>
</tr>
<tr>
<td><strong>Ovariectomy</strong></td>
<td>2.87±1.55††</td>
<td>0.75±0.89††</td>
<td>13.00±2.62††</td>
</tr>
<tr>
<td><strong>Ovariectomy + GSE</strong></td>
<td>5.00±4.30</td>
<td>6.25±2.65** †</td>
<td>22.25±8.75*</td>
</tr>
</tbody>
</table>

Result expressed as mean ± SD, GSE = grape seed extract
* and ** = statistically significant as compared to Ovariectomy group at p<0.05 and p<0.01
† and †† = statistically significant as compared to Control group at p<0.05 and p<0.01

Ovariectomy was responsible for reducing the motility, reflected in number of rears, in squares crossings and also the emotive expressed in defecations (p<0.01). It not a widely accepted opinion that ovariectomy is able to induce the decreasing of motility. Monteiro, et al. (9) assumed that ovariectomy did not affect the number of crossings, but other studies, relate the response to open field test to number hormonal status (10). However, the GSE proved a significant ameliorative effect, the number of defecations and crossings were improved in a significant manner (p<0.05), and distinctly significant manner (p<0.01) respectively.

Even various plant compounds have antidepressant properties; relatively few studies on GSE were done. Therefore, the mechanism remain obscure, but is rational to suppose that this improvement in mental status is subsidiary to general tonic effect provided by the plant compounds, because clinical evaluation revealed a much better condition in treated animals as compared to other groups, including sham operated animals.

In post menopausal women, the impairment of cognitive functions is not only induced by the estrogenic withdrawal, but also with degenerative phenomena inherent to aging process, many related to increased oxidative damage. Diminished efficiency of brain antioxidant systems in elderly rats leads to an intense formation of lipids peroxides (11, 12), carbonyl proteins, reduction of total donor hydrogen ability (11), functional impairment of cholinergic system (12), and consequently, loss of memory (11). GSE prevents, to some extent, these side effects of aging, not as a simple antioxidant only, but as an inductor of the antioxidant enzymes CAT and SOD (12). The inductor mechanism of GSE was further proved on gene expression of antioxidant enzymes in vitro, on HepG2 hepatocarcinoma cell line (13).

**CONCLUSIONS**

On rat ovariectomy model, GSE prevented the plasma lipid peroxidation and improved the cognitive functions, but it did not affect the level of uterine involution and the body weight gain. These findings recommend it as possible alternative remedy against menopausal associated pathology.

**ACKNOWLEDGMENTS**

The product in study was a generous gift of Mrs Ing. Viorica Iliies, President of TC Alsifcom Ltd.

We wish to kindly acknowledge the technical assistance of Mrs Chem. Nicoleta Decea, Laboratory of Oxidative Stress, from Department of Physiology, University of Medicine and Pharmacy “Iuliu Hatieganu” Cluj Napoca.
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